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THE ULTRASTRUCTURAL RESPONSE OF TWO MORPHOLOGICALLY DISTINCT FORMS OF *CLADOPHORA GLOMERATA* TO THE ALGICIDE HYAMINE

MARCH 1994

Ontario

Ministry of
Environment
and Energy

ISBN 0-7778-1461-7

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PIBS 2875

THE ULTRASTRUCTURAL RESPONSE OF TWO MORPHOLOGICALLY DISTINCT FORMS OF *CLADOPHORA GLOMERATA* TO THE ALGICIDE HYAMINE

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ABSTRACT

Two morphologically distinct forms of Cladophora glomerata isolated from the eastern Great Lakes, one from summer and the other from fall, were used in bioassays in a toxicity test of the algicide Hyamine. Ultrastructurally, the two forms exhibited variations in outline of the cross section, cell wall, chloroplasts, thylakoidal membrane arrangements and abundance of starch and lipid bodies. The summer form which predominated over other submerged filamentous algae in creeks, riverbeds and along lake shorelines, was found to be more tolerant of the algicide, showing only apical cell damage at a concentration of 0.5 mg L^{-1} after four days. In contrast, the fall form prevailing late in November, was completely destroyed at this concentration. The impact of the possible existence of more than one genetic form of C. glomerata in the Great Lakes is discussed in view of this species' frequent use in biomonitoring and bioaccumulation studies.

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b) In the cross section of a fall form cell, alongside the appressed thylakoid stacks are many untangled 1-band thylakoids. Plastoglobuli between thylakoid membranes are generally small (scale bar = 1.0 μm).

Figure 4 After exposure to 0.5 mg L^{-1} Hyamine for 4 days, a) the thylakoid stacks which normally wrap around the pyrenoid in the summer form C. glomerata are now broken into short bundles (scale bar = 0.5 μm), and b) the detached thylakoid membranes are broken into small fragments (scale bar = 0.5 μm).

Figure 5 After exposure to 0.5 mg L^{-1} Hyamine for 4 days, a) the 2 and sometimes 1-band thylakoid membranes surrounding the pyrenoid of the fall form C. glomerata are badly distorted (scale bar = 1.0 μm).

b) Similar disorder in the thylakoidal system can be seen throughout the entire reticulate chloroplast. The endoplasmic reticulum also dilates (see pointer) (scale bar = 0.5 μm).

INTRODUCTION

Cladophora, because of its ubiquity, luxuriant growth and capacity to concentrate contaminants, has been widely used as a bio-indicator in studies of nutrient enrichment and water pollution in aquatic systems (Taft and Kishler 1968; Clements 1990; Jackson et al. 1990).

Freshwater C. glomerata, like its marine counterpart, can be subdivided into forms, subforms and varieties based on its morphological characteristics (van den Hoek 1982). In the western Grest Lakes area, Prescott (1982) categorized four forms.

Recent studies have shown that most morphological characteristics of Cladophora, such as apical ramification and cell dimension, are environmentally dependent and should not be the only criteria used for taxonomic classification (Parodi and Caceres 1991). Evans (1974) has discussed the use of fine structure in algal taxonomy.

During the summer of 1991, Cladophora samples brought back from a water quality surveillance programme along the northern shoreline of Lake Ontario and eastern shoreline of Lake Huron (Jackson 1988), were examined with an electron microscope for the measurement of bioavailable metals using X-ray microanalysis. In these samples, we observed significant variations in the fine structure of the Cladophora cells. Using the light microscope, we also encountered a variety of apical ramifications and branching arrangements. Later in the fall, a second set of Cladophora samples was procured from a littoral zone population in Lake Ontario near Toronto; at this time, one fall form prevailed over the remnants of the summer forms. Using taxonomic keys to Cladophora (Prescott 1982), both the summer and fall forms were identified as C. glomerata (L.) Kütz. (Dr. T. Sawa, Botany Department, Univ. of Toronto).

In toxicity studies, many workers have demonstrated through the identification of ultrastructural changes that algae differing in fine structure had different tolerances to

chemicals through the identification of ultrastructural changes (Dodge 1973). In view of the variety of fine structural arrangements in our C. glomerata, it was thus expected that variations might be expected to occur in the ultrastructural response of these to toxicants such as the algicide Hyamine. The objective of this study, therefore, was to determine the presence and the significance of C. glomerata forms in the Great Lakes. Our approach was to choose from our samples two morphologically distinct C. glomerata, one from summer and one from fall, to expose these cells to a series of Hyamine concentrations, and to examine their ultrastructural response to Hyamine through the use of electron microscopy. The ultrastructural changes described in the text were observed in filaments that had not been affected morphologically as determined by light microscopy.

Hyamine is a quaternary ammonium compound used primarily as a disinfectant in swimming pools (Anon. 1976). Maloney and Palmer (1956) reported that quaternary ammonium compounds were excellent chemicals for controlling the growth of Stigeoclonium, also a green filamentous alga. In a preliminary report, we compared the toxicities of a number of chemicals such as dieldrin, carbofuran and algimycin on Cladophora, and have found that Hyamine at low concentrations was the only effective compound in halting Cladophora apical growth without damaging the main branches (Wong and Wainwright 1992).

METHODS

Cladophora test species: the summer and fall form

Two laboratory C. glomerata cultures were used in this study: one was an axenic culture (C. glomerata (L.) Kütz. UTCC #13) obtained from the University of Toronto, and the other, a non-axenic culture isolated from the fall samples. The UTCC #13 culture was originally isolated from the Thames River, Ontario. Prior to the tests, both cultures were maintained in Bold's Basal Medium (BBM) (Hutchinson and Stokes 1975).

The Cladophora samples collected from the summer of 1991 were intended for analysis of metal contamination in cell tissues. Most samples were collected from polluted sites, below sewage treatment plants or in the receiving waters of industrial waste discharges, and the UTCC #13 culture was therefore used as a control. During the months of examination using the electron microscope, we observed strong similarities in both fine structure and cell morphology between the UTCC #13 culture and the Cladophora species predominating in rivers and along lake shorelines. However, while incubating after isolation, the Cladophora filaments were consistently overwhelmed by epiphytes. Because of this epiphyte problem, and the possibility that Hyamine might have a cumulative toxic effect on the field Cladophora samples that were potentially contaminated by metals or organics, we used the healthy UTCC #13 culture to represent the summer form. In addition, the UTCC #13 culture (summer form) and fall form have different reticulate chloroplast arrangements which are not environmentally dependent.

The fall filaments were gathered from a stretch of shoreline in Lake Ontario near Toronto, away from any STP discharges. In late November, the water temperature was 10 °C and were virtually free of epiphytes. During identification of the fine structure, a sub-sample of clean fall filaments remained healthy in BBM solution, and this subsample was used to represent the fall form even though the culture had not yet been purified.

Both the summer and fall form Cladophora filaments were divided into three portions: the first portion was used for morphological identification, the second for electron microscopic examination and the third for toxicity assays with the algicide Hyamine 3500 (Wong 1985).

Morphology

The morphological variations between the two forms of Cladophora were examined according to the morphological criteria recommended by van den Hoek (1963, 1982): thallus organization; apical ramifications; length; width; and length/width (l/w) ratios of the apical, main and branch cells.

Electron Microscopy

Cladophora filaments were fixed in 4% glutaraldehyde (buffered with 0.1M cacodylate acid) for 6 h and postfixed in 2% osmium tetroxide (same buffer) for 1 hour. The plant samples were dehydrated through a graded ethanol series of 30%, 50%, 70%, 90%, 95% and 100%, embedded in 100% Spurr's medium and then polymerized overnight at 70 °C. Thin sections were cut with a microtome and poststained with uranyl acetate and lead citrate. Observations were made using a Siemens ELMISKOP 102 electron microscope.

Algal assays

The toxicity tests with Hyamine 3500 (Rohm and Haas Co.) were carried out in 250 mL Erlenmeyer flasks, each containing 100 mL Bold's Basal Medium (Hutchinson and Stokes 1975). The Hyamine concentrations used in the tests were diluted to give 0.3, 0.5, 0.8 and 1.0 mg L^{-1}, with zero Hyamine concentration as the control. A biomass of 0.5 g wet weight Cladophora filaments was used as the inoculum. Cultures were incubated for four days at 20 °C in a Percival growth chamber at a light intensity of 60 μEin m^{-2} sec^{-1} with a 12:12 LD photoperiod. At the end of the 4th day, part of the Cladophora sample was prepared for electron microscopic examination, while the remaining part was returned to the growth chamber for another 21 days incubation for light microscopic examination.

RESULTS

Morphological differences

In the control, the summer form C. glomerata was thick with branches and branchlets and exhibited acropetal organization. The branches were delicate, comprised of cylindrical cells. As they developed into branchlets, the cells became thinner and tapered off to slightly pointed apical cells (Fig. 1a). The fall form Cladophora in contrast was much coarser, thicker in diameter and firmer in texture. Cell widths were consistent between branches and

branchlets (Fig. 1b) but much broader in main filaments. The branches were slightly incurved (falcate) and the filaments were occasionally interlaced (inset, Fig. 1b). Other comparative details are presented in Table 1.

The fine structure of the summer form C. glomerata cells has been described in a previous study (Wong and Wainwright 1992). Briefly, the multi-layered cell wall consists of alternating microfibrillar and amorphous layers (Fig. 2a). Small vesicles are attached to the plasmalemma. The reticulate chloroplast is made up of individual chloroplasts connected by strands of cytoplasm (Fig. 2a). Among the chloroplasts are pyrenoids; each consists of two electron dense hemispheres encompassed by a starch plate. The thylakoid membranes are double-banded. In stacks, they run along both sides of the starch grain sitting in the middle of the chromatophore as presented in the cross section in Fig. 3a. Plastoglobuli (chloroplast lipids) are large and abundant. Internal to the chloroplasts are the nuclei. Surrounding the nuclei in the perinuclear areas are the Golgi bodies, vesicles and mitochondria. Lipid bodies can be numerous depending on the maturity of the cell. In the central space of the cylindrical cell is the large central vacuole.

Unlike the summer form, the cross section of the fall form C. glomerata cell was neither oval nor spherical but was irregular in shape. Compared to the summer form, the apical cells of the fall form were about twice the volume. These cells had rugged surfaces (inset, Fig 2b), and the cell walls were less dense and less compact. Each cell contained a single chloroplast with long thylakoid membranes forming a continuous, intricate network in the chromatophore (Fig. 2b).

Cross-sectionally, the thylakoidal membranes in the fall form were less appressed. There were few stacks. Many double bands were separated by large interthylakoidal spaces and sometimes the bands reduced to single-band membranes (Fig. 3b). Plastiglobuli were small, starch grains were rare, and mitochondria minute and scattered.

Toxicity tests with Hyamine

The fall form C. glomerata filaments proved to be more susceptible to Hyamine toxicity, despite their robust appearance. After four days, the Cladophora filaments showed no noticeable changes from the control at 0.3 mg L^{-1} Hyamine solution. The fall Cladophora filaments at 0.5 mg L^{-1} sustained damage in the branches, branchlets and in the main filaments, while only the apical cells of the summer form were damaged. At 0.8 and 1.0 mg L^{-1}, the filaments were badly decomposed in both fall and summer form filaments. Thin sections at 0.5 mg L^{-1} were thus singled out for ultrastructural examination.

Ultrastructurally, for both forms of Cladophora, the chloroplasts and pyrenoids appeared to be the organelles most affected. In the summer form, the once tightly packed thylakoid stacks were now broken into small bundles (Fig. 4a). The membranes on the side and at the end of the bundle detached and became fragmented (Fig. 4b). In contrast to the fragmented thylakoid pieces of the summer form, the isolated bands of membranes in the fall form C. glomerata curled and coiled into loops (Figs. 5a and b).

Much less obvious in the fall form were the dilated endoplasmic reticula in the perinuclear areas (pointer, Fig. 5b). There were many huge vesicles. The mitochondria were devoid of cristae.

After 25 days, filaments in the 0.5 mg L^{-1} Hyamine solution were re-examined. With the summer form, we observed no further destruction to the branches and branchlets. Many apical cells survived and were able to regrow (Wong and Wainwright 1992), But with the fall form Cladophora filaments, no living cells remained.

DISCUSSION

There are more than a hundred marine species of Cladophora as documented by van den Hoek (1963, 1982). In freshwater, Rabenhorst (1868) reported only eight species, but if we

include all the species, subspecies, varieties, forms and subforms, there are more than fifty taxa (van den Hoek 1982).

Traditionally, the taxonomy of Cladophora species has been determined by species habitat, and by aspects of cell morphology such as the organization of the thallus and the mode of growth. Unfortunately most of these morphological criteria are not clearly defined, and a number of Cladophora species have been given many different names (van den Hoek 1982). In recent research, studies have shown that environmental factors such as water temperature, air exposure and river flow (Usher and Blinn 1990; and Parodi and Caceres 1991), can alter the morphology of Cladophora, making it difficult to follow the systematics of the genus. This prompted many researchers, in an attempt to circumvent the problems in the taxonomy of the genus, to switch to chromosome numbers (Sinha 1967; Shyam 1990; Parodi and Caceres 1991) or DNA-DNA hybridization (Bot et al. 1991) for identification. There are other techniques apart from these methods, such as the use of hydrogenase (Kesser 1974). In our tests, we approached the problem with the more conservative electron microscopic technique.

Electron microscopy has been available for decades, and there are many well established methods enabling workers to identify fine structure or ultrastructural changes of algal cells (Dodge 1973). Cladophora, a popular bio-indicator in studies of water pollution, has had its fine structure investigated by many workers; for instance, studies have been made of the multiple alternating layers of Cladophora cell walls (Hanic and Craigie 1969), and of the various nuclear phases in mitotic division (McDonald and Pickett-Heaps 1976). Nevertheless, despite the large number of papers written on the ultrastructure of Cladophora cells, there has yet to be an attempt to use fine structural differences in subspecific classification, even though from time to time there have been studies showing structural diversity at the subcellular level of Cladophora cells (Sinha 1967).

In the Great Lakes, although researchers were not that interested in rigorously identifying Cladophora, there were descriptions of nonconforming C. glomerata which may have been

varieties or forms of the species. For example, Blum (1982) described two distinguishable populations of C. glomerata, one above the water line of Lake Michigan and the other below. The population above the water line was referred to as perennial Cladophora, and the one below, as hydrolittoral Cladophora. Apparently, the perennial Cladophora was unusual because it exhibited no summer growth decline, while the hydrolittoral form followed a traditional bimodal growth pattern (i.e., one biomass peak in May and one in September). Another example of the perennial form may be the C. glomerata found by Lorenz and Hendendorf (1982), growing well into late January in western Lake Erie.

In a toxicity study, Whitton (1970) compared the responses of 25 filamentous algal species to metals and found that C. glomerata was the most sensitive. This high sensitivity to contaminants was also observed by Meschkat (1937) and Uspenski (1927) as cited in the Cladophora review by van den Hoek (1963). Conversely, in recent surveys of the Great Lakes, several workers have noted abundant growth of C. glomerata in many contaminated areas. Also, high concentrations of pollutants such as PCB, DDE, Cu, Hg and other metals, have been documented in Cladophora tissues (Anderson et al. 1982; Jackson 1988; Jackson et al. 1990). These contradictory observations could have only been the result of either C. glomerata adapting to polluted waters (Clements 1990) or it having many forms, some more resistant than others. In fact, we have little information on possible subspecific taxa of C. glomerata in the Great Lakes, and even if there are forms, we have no idea how they might respond to contaminants. However, at the species level, there have been reports on differences in internal nutrient concentrations, for instance, Gordon et al. (1981) have shown that Cladophora aff. albida (Huds.) Kütz. has a high phosphorus critical concentration of 3.3 mg g^{-1} (tissue concentration) when compared to 1.3 and 1.6 mg g^{-1} determined by Gerloff and Krombholz (1966) and Wong and Clark (1976), respectively.

Among our C. glomerata samples from the eastern Great Lakes, aside from morphological variation, we observed forms containing a reticulate chloroplast of different arrangements like those described in the text, forms exhibiting three nucleoli in the nuclei, forms with elongated chloroplasts and forms with minute mitochondria. For different types of structural

arrangements (of plastids or nuclei, for example), researchers have shown that algal cells such as Chlorella and Chlamydomonas have different ultrastructural responses to toxicants (Dodge 1973). Organic compounds, such as hydrocarbons and herbicides, are ordinarily indiscriminatory in their attack on algal cells. With Hyamine, on the other hand, the primary target is the chloroplasts (Wong and Wainwright 1992). The algicide's antiplastidial action enables us to distinguish ultrastructural changes in the reticulate chloroplast of the summer and fall forms of C. glomerata cells.

Ultrastructurally, the Cladophora cells of these forms exhibited dissimilarities in cell shape, cell wall, chloroplasts, sizes of the plastoglobuli and mitochrondria, abundance of starch grains and lipid bodies, and thylakoidal membrane arrangements in the chloroplast(s). These fine structures of the fall form Cladophora cells were much more sensitive and collapsed faster than the summer form cells in the presence of Hyamine solution. Although the chloroplasts (or more specifically, the thylakoids) deteriorated in different ways, it was not certain whether the cell walls and/or amounts of lipids, starch grains and polyphosphate bodies also played a part. Considering that lipids and polyphosphate bodies are the sites where metals and organic toxicants can be bound and localized (Sicko-Goad et al. 1989; Rachlin et al. 1982), the storage of large amounts of these products in summer form cells could explain why summer form filaments are so widespread in many of the more polluted waters.

Now that we have determined that in the Great Lakes, we may have C. glomerata forms that resist toxic chemicals, produce little reserved food, are unable to accumulate high concentrations of contaminants, and tolerate high or perhaps low temperatures, we should at least ensure that the C. glomerata used in biomonitoring samples are of the same form before we commit ourselves to future bioaccumulation studies in the Great Lakes.

ACKNOWLEDGMENTS

We would like to thank K.H. Nicholls for reviewing the manuscript and Dr. T. Sawa for his help with the taxonomy of the Cladophora species.

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Table 1 Morphological variations between the two forms of C. glomerata.

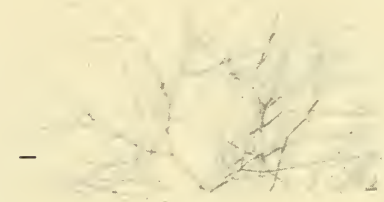
summer form (UTCC#13)	fall form
Thallus organization	
acropetal	acropetal
Ramification	
profuse	sparse
Branches	
straight	slight incurved
cells decrease in thickness towards branchlets and apices	even width
slightly pointed tips in apical cells	bluntly rounded tips
mostly pseudodichotomous branching with occasional trichotomous	many trichotomous
main filaments separate	many interlaced filaments
Filaments	
can grow to meter long in fast moving water	short
barrel shape akinetes (dark green)	unobserved
few intercalary cells	many
Average cell length, width, length/width ratio (mean ± S.D.)	
apical	
length 350 ± 67 μm	280 ± 21 μm
width 28 ± 4 μm	70 ± 9 μm
l/w ratio 12.5 ± 1.3	4.0 ± 0.8
branch	
length 390 ± 82 μm	350 ± 54 μm
width 56 ± 12 μm	70 ± 14 μm
l/w ratio 7.0 ± 4.2	5.0 ± 3.0
main filament	
length 700 ± 125 μm	460 ± 75 μm
width 140 ± 15 μm	110 ± 36 μm
l/w ratio 5.0 ± 0.8	4.2 ± 2.3

Figure 1 Light micrographs showing a) summer form C. glomerata and b) fall form C.glomerata which carries occasional interlacing filaments (inset). (Scale bars = 200 μm).

Legend:

C	chloroplast
CV	central vacuole
CW	cell wall
ER	endoplasmic reticulum
L	lipid
M	mitochondrion
N	nucleus
P	pyrenoid
OB	plastoglubuli (osmiophilic bodies)
Pl	plasmalemma
S	starch
Sp	starch plate
T	thylakoids

1a



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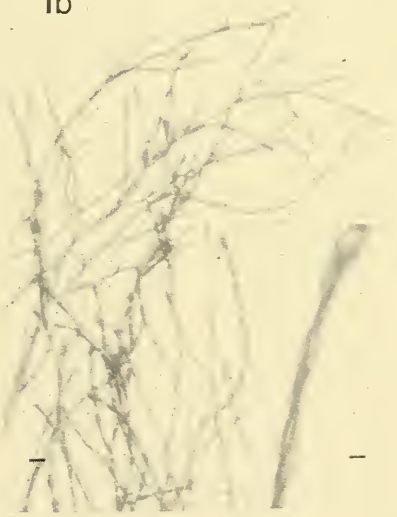

Figure 2 Electron micrographs illustrating a) a network of individual chloroplasts in a longitudinal section of the summer form C. glomerata (scale bar = 1.0 μm), and b) a portion of the single reticulate chloroplast in the cell of the fall form C. glomerata which also exhibits a rugged outer cell wall surface (inset), (scale bar = 1.0 μm).

Legend:

C	chloroplast
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N	nucleus
P	pyrenoid
OB	plastoglubuli (osmiophilic bodies)
Pl	plasmalemma
S	starch
Sp	starch plate
T	thylakoids

Figure 3 a) Stacks of fused thylakoid membranes run along both sides of a large starch grain sitting in the middle of a chloroplast captured in the cross section of a summer form cell (scale bar = 0.μm).

b) In the cross section of a fall form cell, alongside the appressed thylakoid stacks are many untangled 1-band thylakoids. Plastoglobuli between thylakoid membranes are generally small (scale bar = 1.0 μm).

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Pl	plasmalemma
S	starch
Sp	starch plate
T	thylakoids

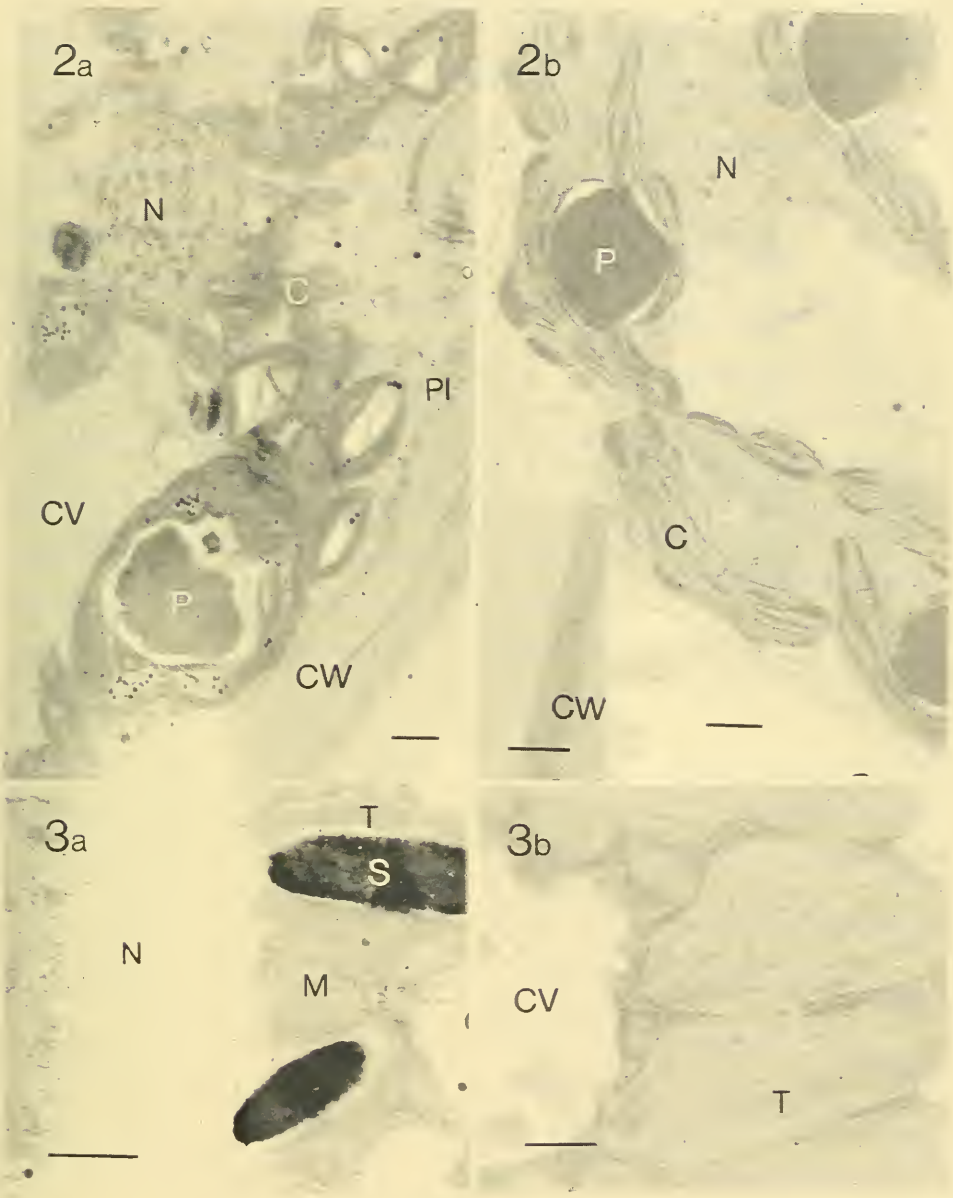

2a
N
C
Pl
CV
P
CW
2b
N
P
C
CW
3a
T
S
N
M
3b
CV
T

Figure 4 After exposure to 0.5 mg L^{-1} Hyamine for 4 days, a) the thylakoid stacks which normally wrap around the pyrenoid in the summer form C. glomerata are now broken into short bundles (scale bar = 0.5 μm), and b) the detached thylakoid membranes are broken into small fragments (scale bar = 0.5 μm).

Legend:

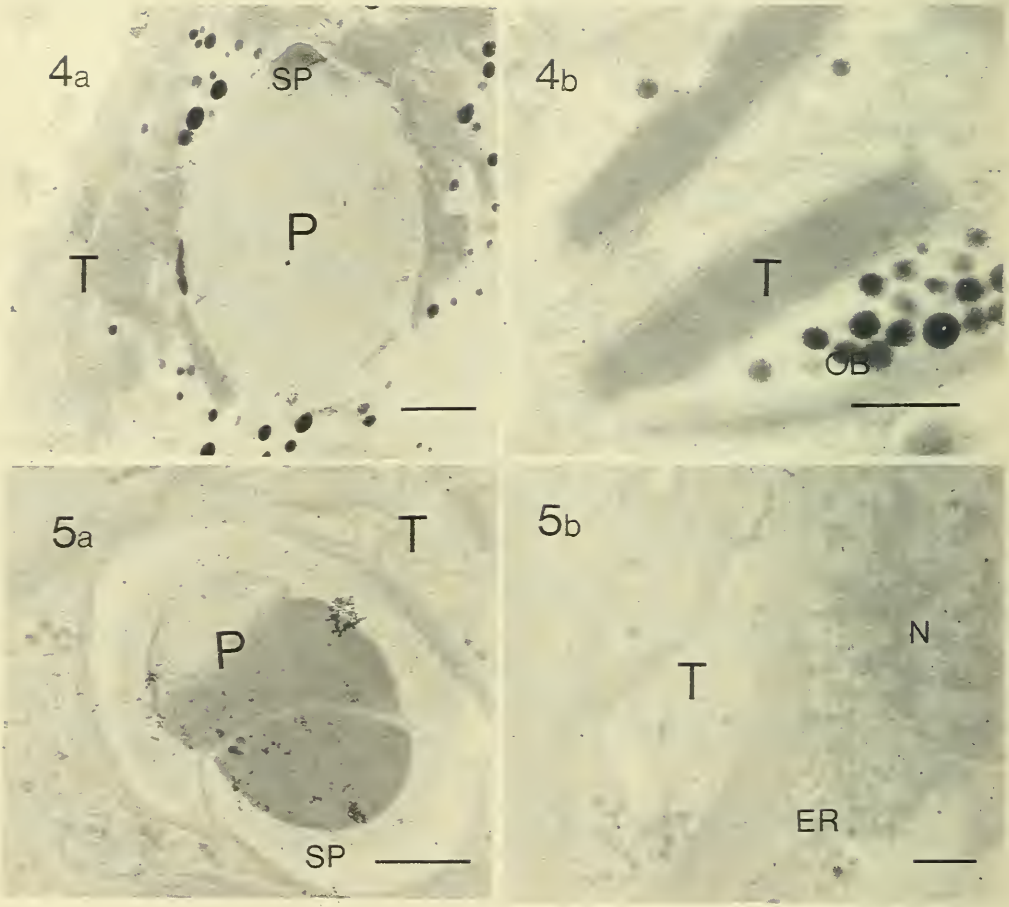
C	chloroplast
CV	central vacuole
CW	cell wall
ER	endoplasmic reticulum
L	lipid
M	mitochondrion
N	nucleus
P	pyrenoid
OB	plastoglubuli (osmiophilic bodies)
Pl	plasmalemma
S	starch
Sp	starch plate
T	thylakoids

Figure 5 After exposure to 0.5 mg L^{-1} Hyamine for 4 days, a) the 2 and sometimes 1-band thylakoid membranes surrounding the pyrenoid of the fall form C. glomerata are badly distorted (scale bar = 1.0 μm).

b) Similar disorder in the thylakoidal system can be seen throughout the entire reticulate chloroplast. The endoplasmic reticulum also dilates (see pointer) (scale bar = 0.5 μm).

Legend:

C	chloroplast
CV	central vacuole
CW	cell wall
ER	endoplasmic reticulum
L	lipid
M	mitochondrion
N	nucleus
P	pyrenoid
OB	plastoglubuli (osmiophilic bodies)
Pl	plasmalemma
S	starch
Sp	starch plate
T	thylakoids


4a
SP
P
T
4b
T
OB
5a
T
P
SP
5b
N
T
ER